Message Information

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From steven pedigo <stevenosei@msn.com>

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cc

Subject Permit issuance for Deep Horizon spill and Pre approval

Message Body

To All involved in the RRT VI or members of the RRT VI,

Attached are several documents regarding the importance of having the RRT immediately issue the necessary permit for the non-toxic product called OSE II to be implemented as a cleanup tool for BP's Deepwater Horizon oil blow out response. We are not sure everyone received this section of the email,

this is to make absolutely sure.

1) The first document is the scientific basis and reasoning per the Oil Spill Selection Guide, established by Regions III and IV, to immediately approve OSE II for the cleanup response to this spill.

2) Several other documents, including correspondence between the OSEI Corporation and the US EPA that clarify
concerns that have been expressed and resolved.

This package of information addresses and should fully resolve every possible question an RRT official might have
regarding this issue. I would appreciate it, if anyone who is associated or a member of the RRT VI who was not emailed this request, the EPA, or Coast Guard member of RRT VI will copy the entirety of this email and its contents to any member not in receipt of this information.

I await your quick response and issuance of the permit to the OSEI Corporation for the application of OSE II on the Deepwater Horizon oil. The Coast Guard has requested its immediate implementation and, with the attached package of information in hand, there is now no viable or scientific reason why it should not be immediately authorized for use.

Sincerely,
Steven Pedigo


EPA_RRT_counter_for_demonstration_decision_July 1st, 2011.doc14 toxicity test summaries July 2011 .doc

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INTRODUCTION

Since 1989, despite voluminous and incontrovertible scientific evidence demonstrating the extraordinary and swift effectiveness of the non-toxic first-response, oil spill cleanup method called OSE II, the product has been arbitrarily frozen out of the US navigable water clean up business by the US EPA, NOAA and other federal agencies represented in the EPA's Regional Response Team (RRT). This group has created a framework of conditions that support an existing monopoly for the Exxon Corporation's product Corexit 9527a. In May of 2010, when the EPA demanded that BP find another cleanup method for the Deepwater Horizon than Corexit 9527a, the RRT approved in lightening speed (within 24 hours) BP's requested substitute - Exxon's other product, Corexit 9500, without regard to its toxic adverse effects, and/or its lack of value to the BP Deepwater Horizon oil cleanup response.

The use of the two Corexit products in this disaster has, predictably per their labels and official Material Safety Data Sheets, exposed them to the broad public as being the horrifically toxic chemicals that they are, and this fact has been underscored by the test results of numerous independent scientists.

EPA/NOAA RATIONALIZATIONS

OSE II (the enzymatic product with no microbes in it which is already on the official EPA National Contingency Plan for oil spill cleanup) has had repeated requests from the injured Gulf States for its implementation as a non-toxic, first-response cleanup method, but the EPA/NOAA have ignored these requests, and/or used false, non-scientific justifications for arbitrarily stopping the use of this product, which is the world's most experienced and effective, hydrocarbon-based, cleanup tool.

The first specious reason for not allowing OSE II to be implemented in the Deepwater Horizon disaster was expressed by Sam Coleman (Director of the

Superfund Division, EPA Region 6, and the EPA's RRT6 representative). Despite the fact that as early as 1996 the EPA insisted that OSEI Corporation prove it was not a sinking agent, and the subsequent test results are in EPA's files that clearly demonstrate that OSE II operates exactly opposite to a dispersant and/or sinking agent, Coleman stated that they "were worried OSE II would sink oil," necessitating the repetitive process of explaining, once again, how groundless his concerns were.

Additionally, as recently as March of 2011, tests on OSE II were completed by BP's Dr. Tsao at LSU laboratories, while in close communication with the members of RRT 6, once again proving to the EPA and Sam Coleman that OSE II does not sink oil.

The next justification the EPA/NOAA used to prevent OSE II's implementation was that they "were worried that OSE II would grow too many indigenous bacteria and that this would somehow create a bigger problem after the oil was digested and broken down." It is important to note that NOAA is the scientific advisor to the EPA. It was astonishing to receive this statement by a scientist from NOAA because it shows a complete ignorance of the most basic factors of bioremediation and microbiological processes. Most first-year biology students learn that any eco system can only sustain that amount of life supported by readily available food. Once the food is depleted, that eco system will no longer sustain the same amount of life, and, in the example of bio-stimulation of indigenous microbes, the surplus of microbes simply die back to their normal background levels after the oil is digested, with no negative side effects to the environment of any kind.

ARE EPA/NOAA OFFICIALS ACTUALLY LOOKING FOR NON-TOXIC SOLUTONS?

EPA/NOAA are responsible for protecting the environment. They have purportedly been in the process of diligently researching the various potentially viable non-toxic solutions for cleaning up the oil blowout. All the necessary information from tests done on OSE II at the request of the EPA over the past 21 years, plus the current tests completed in March by BP at LSU, plus information regarding the over 16,000 real-life oil spill cleanups successfully performed by OSE II, with not one negative side effect ever reported, have been provided to the EPA/NOAA as a part of this allegedly sincere vetting process. Had the EPA/NOAA honestly reviewed the OSE II information, including pictures of the over 5,000 gallon significant crude oil

spill cleaned up with OSE II for Texaco in a closed, large pond, they would have seen the fact that OSE II causes the oil to float until it is converted to water and CO2. They would also have seen the natural process of steps that occur when OSE II is applied to an oiled environment: 1) bacteria grow on the oil's surface, 2) clump up as the food source diminishes, and then 3) return to background levels once the crude oil/food source had been depleted. They would have also seen that the use of OSE II does not harm the flora and fauna, and, in fact, protects the marsh grass, birds, fish, turtles, snakes, and the rest of marine and wildlife, and prevents migratory birds from getting coated with oil and dying from exposure. See link http://osei.us/photoalbums/crude-oil-spill-cleanup

It is very apparent that either these officials did not bother reviewing OSE II's easily-accessed public information on our web site which we have referred them to repeatedly in order to help them make the best clean up response decisions, or that, if they did review the information, they have entirely other agendas than genuinely wanting to clean up the Deepwater Horizon disaster.

ANOTHER UNWARRANTED CONCERN

Another verbal pretext that was given to Sanford Phillips of LA DEQ to justify why EPA/NOAA was refusing to allow LA DEQ to implement OSE II for this disaster was stated by Charlie Henry of NOAA. Henry is NOAA's Lead Scientific Support Coordinator for the Deepwater Horizon Response. Henry made a blanket statement that "no product will be used that contains surfactants". Again, this was a strikingly uneducated statement coming from a NOAA official as it showed complete ignorance of the predictable processes Mother Nature utilizes to clean up an oil spill. Surfactants are a natural part of that process. I subsequently thought I had put this matter to rest with an explanatory letter to Charlie Henry, which I copied to the other senior EPA and RRT officials; however, as though that letter was never received or read, DOC and NOAA officials, once again, made the same groundless statement several months later as their most recent justification for preventing the implementation of OSE II. The toxicity test results the EPA has for OSE II (of which, a predominant number were performed by the EPA themselves), showing that OSE II, as a product, is completely non-toxic, proves that the type of surfactant it contains is of no concern. Despite this, the repeated presentation of the pertinent scientific facts related to this have been ignored by EPA/NOAA.
Letter attached.

On the other hand, BP's Dr. Tsao relayed to us that the RRT claimed that they agree with the use of bioremediation technology, "as long as the products don't contain a surfactant." Of note is that *Corexit contains 4 different chemical surfactants.* Apparently, however, that was not an issue of concern when they rushed through the permits for its use despite the fact that one needs only to read Corexit's label and MSDS sheets to know that it is lethally toxic to people, flora and fauna.

Again, the unfounded justification for not allowing OSE II to participate in the BP/LSU field demonstration that was to occur once products had proven themselves in the LSU lab as being potentially viable solutions, was that it contains a chemical surfactant. If those responsible for vetting alternative, non-toxic solutions to cleaning up the Deepwater Horizon disaster have actually read any of the documentation we supplied, or seen any of the toxicity tests easily accessed on OSEI's website under the "Technical Library" section, then they know that OSE II is completely non toxic.

For those who have not read it, and/or are interested, the results of 14 different toxicity tests are attached to this letter: 10 salt water species, 3 fresh water species, and one water flea. They show, overwhelmingly, that OSE II is safe for marine species, the environment and people. So, again, the fact that OSE II has a surfactant in it is completely inconsequential as far as the safety and effectiveness of implementing it. Using this as an excuse to justify preventing its implementation is scientifically illogical.

The chemicals that 40 CFR outlaws and which cause a product to be unsafe and prevent it from being approved for inclusion on the EPA's NCP list, are chlorinated hydrocarbons and trace elements. OSE II does not have any of these and it has been on the NCP list for many years. In addition to voluminous scientific test proof, it has been proven empirically to be non-toxic to marine species and humans since, as a demonstration, OSEI staff have actually ingested it on TV and it has been utilized by the US Navy in areas with abundant marine life nearby, including dolphins and whales, and had absolutely no negative impacts on any species.

The EPA NCP testing has substantiated that OSE II has a defined endpoint: it converts oil to CO2 and water. BP's recent LSU test on the combination of Louisiana sweet crude oil mixed with Corexit dispersant proved OSE II was the most effective product at remediating the PAH's in the oil, which are the most toxic and persistent components of crude oil per the US EPA.

The object of any spill response is to lessen the toxicity to the environment in order for living organisms to be able to survive. The desired result would be to clean up 100% of a spill, and OSE II has proven it does exactly that over 16,000 times on both fresh and salt water spills, and wherever hydrocarbon-based material is spilled. No other product in the world has the first response capabilities with the swift and financially viable desired outcomes of OSE II: it is able to address 100% of the spill, limit a spill's environmental impact, protect natural resources, and return the area involved to pre-spill conditions in usually less than 2 weeks, once it comes in contact with the oil, and not usually more than 4 weeks. OSE II is a *sole source* clean up product, and never has there been a more vitally important time to get it implemented then on the massively catastrophic situation that currently exists in the Gulf of Mexico as a result of the on-going Deepwater Horizon disaster.

<u>There is no legitimate scientific reason not to use OSE II immediately.</u>

<u>EPA IGNORES NOAA'S ALREADY ESTABLISHED GUIDELINES</u>

It is important to note that the NOAA selection guide, established by the RRTs 3 and 4 in cooperation with the NRT and paid for by the US Coast Guard, provides useful tools in deciding which product(s) to use for the cleanup of an oil spill. These guidelines are based on toxicity and ability.

Clearly stated on page VIII under "Basic Reasoning" are the following parameters:

1. **Decide if applied technology might provide value.**
   When one looks at this guideline in relationship to the choice of chemical dispersants used in the Deepwater Horizon, neither of the Corexits added anything of value; in fact, they exacerbated the problems of the BP spill by adding substantially more toxicity to the already toxic situation caused by the oil, and spread it exponentially further throughout the marine environment. On the other hand, when looking at whether or not OSE II, if applied, provides value, one finds that it has a <u>substantiated end point</u> of CO2 and water and prevents oil from unnecessarily contaminating additional areas (the water column below the surface, the seabed, the beaches and the marine life/seafood). The combination of the latter with the fact that it is non-toxic, gives OSE II considerable value.
2. **Decide if the OSC has the authority to use it within its useful time frame.**

This specifically pertains to both Corexits since they cannot be used on weathered oil, and, therefore, must be applied to the oil within a matter of a couple of days or less, after it has released into the environment.

On the other hand, OSE II has no time frame limits and can be used as a first-response tool *and* at any point after oil has escaped into the environment. It works equally well whether it is fresh oil or weathered. There are no time limitations whatsoever. Additionally, because it is already on the NCP list, it can be legally used by the OSC immediately.

3. **If so, can it be here in time?**
   The OSEI Corporation keeps enough OSE II on hand to clean up 1 million gallons of oil, or hydrocarbon-based material, on an immediate basis and can rapidly ramp up manufacturing to meet any requirement, in multiple countries, and has. We have been fully prepared to deploy in response to the Deepwater Horizon disaster since the beginning of the incident. Yet, as noted above, the EPA has actively prevented it.
4. **If so, does it have application requirements that exceed the window of opportunity?**
   As stated earlier, both Corexits have narrow time windows of opportunity for application, while OSE II has no time application requirements that exceed any window of opportunity; it can be used as a first and only response method, and has been used and tested and used on all types of oil and hydrocarbon-based material, both fresh and weathered, with no limitations.
5. **If not, does it have unacceptable environmental requirements, health, and safety risks associated with its use?**
   As can be readily seen on their labels and Material Safety Data Sheets, both Corexits have egregious health and safety risks. To protect responders, one must wear chem suits and full face respirators. Their EPA toxicity tests show them to be extremely toxic. If spilled, they are to be cleaned up as a hazardous material. And, yet, the EPA has allowed them to be spread in massive amounts throughout enormous areas of the Gulf waters, even though they had a known history of severe adverse health problems in regards to responders in the Valdez spill. Corexit dispersants have no defined or substantiated end point. However, per the Woods Hole Oceanographic Institute tests just completed in March of 2011, it has been proven that both Corexits cause oil to linger longer in the water column and sediment and actually slow down the natural biodegradation processes even more than if no response method at all had been used on the blown out oil.

Conversely, as mentioned above, OSE II is so non-toxic it has been ingested on TV demonstrations to show its safety, and we have videos and numerous photos of contractors and OSEI personnel washing their hands in it with no adverse side effects over the last 22 years. The numerous toxicity tests on the OSEI web site at www.osei.us, under "Technical Library" and the toxicity tests attached show OSE II to be virtually non-toxic. In direct contrast to both Corexits, OSE II has a predictable, substantiated result/end point: CO2 and water, and it achieves this result, regularly, in less than 2 weeks, but usually not more than 4.

6. **If it has special operational requirements, is there an identified specialist (technical contact) who can provide timely advice on its effective use?**

   Both Corexits have limited windows, and need special, costly equipment to apply it in order to protect responders. However, an example of the ease with which OSE II can be applied is that the OSEI Corporation showed some Louisiana fishermen how to measure and apply OSE II effectively in less than 15 minutes of training. And no hazardous material suits or respirators or hazardous material training were required. All equipment needed to apply OSE II is readily available, and quickly obtainable. There are numerous OSEI Corporation associates that are available on immediate notice to consult on spills, as needed.

These essential NOAA guidelines have been ignored by the RRT 6. It is obvious that none of these points were honestly considered when choosing what products to use for the Deepwater Horizon oil cleanup response, and it is the lack of its use that has resulted in the extraordinarily inadequate and disastrous consequences.

The guide also includes specific instructions related to what should be considered regarding toxicity levels when choosing which products to use. Both Corexits completely violate the guide's rules related to toxicity, while OSE II fully aligns with its toxicity guidelines.

BP's "BioChem Strike Team" testing at LSU has now shown that OSE II reduced more of the toxic components of the oil (PAH's) over any other product tested by a significant value; per the results that were sent to me, it appears to have been over 65% better than the next best product.

THE EPA'S INTENTIONS TO HONESTLY TEST FOR NON-TOXIC, ALTERNATIVE SOLUTIONS TO COREXIT ARE SUSPECT

A testing process began in June of 2010 ostensibly to isolate non-toxic, better alternatives to Corexit. The stated protocol was that, after successful lab tests on several alternative products were conducted at LSU, final tests on Deepwater Horizon oil in the field were to be the ultimate deciding factor for EPA/RRT approval for their implementation.

After stringing along for over a year some companies with alternative products by slowly doing tests in a lab at LSU (tests that should have taken 2 to 4 weeks took 9 months), the EPA arbitrarily decided, on April 14, 2011, not to follow through with the field demonstrations although they did not inform us of their decision. LA DEQ, in an effort to prevent their state's natural resources from continued destruction by Corexit, went to battle to get the field demonstrations done and the EPA changed their position and agreed, on April 21, 2011, to allow a field demonstration, but with one caveat: they would (once again) not use any product that contained a surfactant. As OSE II is a product of those being tested, that contains a surfactant, this was obviously intended to prevent OSE II from being included in the field tests. As clearly explained above, and to the EPA a few weeks prior, refusing to allow OSE II to do the field tests because it has a surfactant has no scientific validity and is baseless as a justification for not using OSE II. However, instead, they chose four of the ten products tested by BP in the LSU lab for the field demonstration that they knew would not work.

The LSU tests and their own prior EPA tests show these products to be very poor at reducing the most toxic components of the oil, the PAH's. Despite the fact that OSE II's results in the LSU lab tests were irrefutably better than any other product at handling the PAH's, the EPA/RRT decided not to include it in the field demonstrations. The EPA has tested 3 of these products and OSE II in the past, in an estuarine environment (see attached EPA estuarine test) (also see attached EPA fact sheet), and OSE II was the only product that proved it could work.

The fourth product has a toxicity value demonstrated to kill 50% of Menidia[1] in 96 hours when they come in contact with 25.33 parts per million

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[1] **Menidia** beryllina (a small fish) are the current EPA-approved marine vertebrate used in both acute and chronic toxicity testing.

of the product, and 50% of Mysidopsis[2] die within 48 hours when coming in contact with 25.33 parts per million.

The fourth product's EPA toxicity tests show it to be as toxic as the two Corexits, while only reducing 10% of the toxic part of the oil, the PAH's, meaning it is relatively valueless, per the NOAA guidelines and common sense.

The EPA had to have known that all 4 products chosen would fail the tests, based on their earlier tests, when they chose them to be applied in a field demonstration. The only logical reason for them doing this is to help them to justify their use of Corexit, ie, "We tried bio remediation and it didn't work." I clearly pointed this out to them in a letter to LA DEQ/RRT shortly after their decision to only test these 4 products in the field came out, and, again, presented the reasons why OSE II should be allowed to participate in the field tests. A few days after my letter was received, Dr. Tsao notified OSEI, and presumably the other bio remediation companies, that the RRT/EPA had, once again, just changed their mind and decided not to run the field tests at all, with no reason given. The EPA has certainly been consistent over the past 21 years in its effort to thwart the implementation of OSE II.

OSE II is the only product the EPA tested in the estuarine environment that showed promise, and, based on OSEI's long history with the EPA, I can only assume that the reason they arbitrarily stopped the field test was to prevent OSE II from demonstrating how effective it would be in completely cleaning up the estuarine environment. In the earlier EPA test done in an estuarine environment in 2002, OSE II had activated the natural bioremediation process when none of the other products had shown any positive results. At that point, the EPA arbitrarily decided to stop the tests and not allow them to complete; again, with no reason stated.
The EPA and NOAA have again repeated the statement they would not allow a product with a surfactant in an RRT meeting and put it in writing in a Coast Guard RRT letter. And yet, as explained above, they have not only allowed the use of Corexit for 22 years, which has surfactants, but have allowed it to be the only product with "pre approval" status, meaning when an oil spill happens, the responsible party does not have to get a permit to immediately begin using it. It also means they have no other option, initially, when there is a spill, because the EPA has never allowed any other product to be given pre- approval.

There are different types of surfactants. OSE II has safe, non-toxic bio surfactants/surfactants, and Corexit has toxic surfactants. Yet the EPA does not disqualify Corexit. So, to say that the reason OSE II is not being allowed to be utilized in the Deepwater Horizon disaster, or even demonstrated in a field test because it has a surfactant is disingenuous in the extreme.

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[2] Mysid shrimp, also standardly used in toxicity testing

The EPA has defamed the OSEI Corporation's product, OSE II, through the use of scientifically baseless excuses to stop its use, spreading the false impression to others not informed about OSE II that there is something wrong with it and/or that it does not produce the results it has fully proven to produce. The EPA/NOAA and other members of the federal government on the RRT have used baseless concerns, statements that defy all the tests they have to hand in regards to OSE II: their own successful use of OSE II on the Osage Indian reservation, the numerous demonstrations of OSE II on the OSEI web site under "News Videos" for the BP spill, photos showing OSE II's exact process on a crude oil spill for Texaco (entitled "Crude oil spill" under "photos" on the OSEI Corporation web site), a 223 page technical library on our web site with numerous efficacy, toxicity, and other tests to try to overcome the EPA's arbitrary hurdles for the past 22 years. And yet they still continue to make statements that have no scientific basis, which the OSEI Corporation can discredit easily with either test results, videos, photos, or experience.

It would be easy to make some rather snide comments about all of this because refusing to allow the use of OSE II "because it contains [non-toxic] surfactants" is comparable to saying "We won't allow the use of OSE II because it has water in it." This situation would be laughable if there weren't so many people's lives being destroyed by the inadequate, yet still reversible, cleanup response and the broad scale destruction of the environment and marine life of the Gulf wasn't being so negatively impacted. The fact that the EPA/NOAA and other government officials are violating their oaths of office, their charters, and the Clean Water Act by continuing to act in this manner places them in a very untenable position.

EPA CLAIMS TO US CONGRESSMAN THEY HAVE NO PROTOCOL FOR THE USE OF BIOREMEDIATION

On Thursday, June 17, 2011 a senior representative from the EPA stated to a US Congressman that the EPA has no protocol for the use of bioremediation. In fact, if you go to 40 CFR part 300 subpart J, you will see under "Bioremediation" there is nothing there; the page is blank.

However, OSEI obtained in 1992 the EPA's formal Bioremediation protocol, which was completed after extensive, taxpayer-funded testing. We are in the process of locating that in our warehouse archives of over 22 years of information and documentation from the EPA and other federal agencies. In the meantime, attached is the protocol developed by the EPA in conjunction with RRT VI (the lead RRT for the BP Deepwater Horizon blowout). The attached document is a copy of the last draft before the final one was completed. The protocol document was completed in January of 1992 and is written on EPA's letterhead.

The document tracks similarly with the dispersant protocol, except it pertains to bioremediation. This document has existed for approxi-mately 20 years, however the EPA is now denying that it exists, and it has been left out of the Code of Federal

regulations. It is interesting that the completion of the document was during the same period the EPA/NETAC developed the NCP listing protocol, as well as the open water testing procedure for bioremediation products, and the monitoring program for bioremediation products. This document was shelved at the same time Exxon's attempt at a bioremediation product (Inipol EAP 22) was proven to be ineffective and very toxic. Chemically it is basically the same as Corexit with added nutrients.

There is a means and a procedure to use OSE II/bioremediation on a spill, which the EPA has not acknowledged or utilized, despite the fact that the magnitude of this BP disaster calls for every effective tool possible.

EPA VIOLATES STATES' RIGHTS

As there has been, since the beginning of this disaster, a safe, effective means to protect the natural resources and people of the Gulf from the onslaught of toxic oil and the unnecessary use of toxic dispersant, the EPA and NOAA as well as the other federal agencies involved, have violated the Gulf States' Constitutional right to protect their natural resources and the health, safety and welfare of their citizens, forcing these people to endure hardships that were and continue to be preventable by simply granting the States' and BP's requests to utilize OSE II.

Representatives from the State of Louisiana had OSE II's information thoroughly vetted by May 2010 and stated that they had come to the conclusion that OSE II had merit. Some of these same people sit on the RRT and on the EPA's science panel. Governor Jindal attempted to have OSE II demonstrated on Chandelier Island on May 6, 2010, the day the oil first reached the Louisiana barrier islands, but the EPA stopped the demonstration from occurring and sent a veiled threat, through Dwight Bradshaw of the RRT to me, stating that if I followed through on Governor Jindal's request for the field demonstration of OSE II "there would be consequences." The RRT became culpable on that day for all the subsequent damages to the Louisiana coastline.

A SUCCESSFUL FIELD DEMONSTRATION OF OSE II ON DEEPWATER HORIZON OIL HAS BEEN PERFORMED

The Waveland Beach, Mississippi demonstration with Region IV EPA officials present should have alleviated all concerns in regards to OSE II, when you take into consideration the numerous toxicity tests performed on OSE II, the numerous efficacy tests, the EPA NCP tests, and now BP's Deepwater Horizon oil spill test at LSU.

How the Waveland Beach demonstration came about was that Mississippi State Senator Gollot ordered OSEI staff and the Mississippi DEQ to find a place to perform a field demonstration of OSE II. They decided to do it on a beach and in a marsh area

of Waveland Beach. RRT 4 personnel and others were notified of the time and place. The EPA representatives from RRT 4 showed up at the demonstration but, for some reason, started to leave before it was completed. As they were leaving, they told Mark Rettig, an OSEI associate, there was "no way RRT would allow any non-indigenous bacteria to be used in their Gulf waters." When Mark told them that OSE II doesn't have any microbes in it, they became more interested and decided to stay for the full demonstration.

There were about 50 people there, including Senator Gollot and one other state senator, EPA reps from RRT 4, several officials from Mississippi Bureau of Marine Resources (BMR), several officials from MS DEQ, and several BP contractors as well as several media outlets. The DEQ reps not only observed, but they participated in laying out the geographical application area. The area was partitioned and isolated by booms so that the fate of the oil, once it came into contact with OSE II, could be accurately demonstrated.

The demonstration was done. All in attendance saw OSE II being applied by a simple backpack spray apparatus onto a sandy beach area and a marsh grass area with the protective boom around it. All attendees witnessed the successful first stages of OSE II on BP oil laced with Corexit and which had soaked into the sandy beach and was adhering to the marsh grass. They saw that it took less than 5 minutes for the oil to lift off the sandy beach and the grass. In about 5 more minutes the oil broke into such small particles it began to be difficult to see. Within 2 hours it was very difficult to see any part of the oil at all. It floated on the surface until it was completely remediated. Some of the attendees returned 5 days later and no trace of oil could be found.

Also in attendance for the first day's demonstration was ABC News, who captured the entire demonstration on video and aired it on a local news program later that day.

Note: The EPA has never acknowledged this successful demonstration of a non-toxic product on BP's oil, other than to repeatedly imply that it wasn't legal to do this demonstration. I have had to repeatedly point out to them that MS DEQ and Mississippi State Senator Gollot requested and authorized it; that EPA officials were there and witnessed it, and that at the beginning of the demonstration Senator Gollot openly challenged the officials there to stop the demonstration if they had a problem with it, and that no one stepped forward.

This was the first time during the Deepwater Horizon catastrophe that OSE II had an opportunity to prove in a live field test on a Gulf sandy beach and marsh that what the earlier LSU tests from 2009 as well as the EPA/NETAC tests from 1992 showed would play out in this type of environment. Despite the success of the test, the RRT/EPA never acknowledged or acted upon it. [Go to http://osei.us/819 to view the WLOX news program about the OSE II demonstration at Waveland Beach.]

If there was a sincere desire to clean up the contaminated waters and shoreline, this demonstration should alleviate any possible concerns because, after 11 months since the demonstration, the protective booms were removed and the marsh grass is completely free of oil and shows no signs of stress or deterioration from the spill. The sandy beach area where OSE II was applied was dug down into and there were

no tar balls or visible oil residue. Just 25 yards away, as of June 15, 2011, on the other side of the concrete drainage area you can dig down into the sand and discover tar balls and oil residue. See the pictures below that show the difference in the EPA-allowed response (Corexit) and the use of OSE II on the sandy beach after 11 months.

The following pictures show the marsh grass at Waveland Beach, Mississippi where OSE II was applied. Notice how the grass shows no distress and is completely free of oil. Then compare this to the pictures a year later showing how the area not treated with OSE II has been negatively impacted by the EPA-authorized response method. The marsh grass shows distress and deterioration. These pictures were all taken on June 15th, 2011, 11 months after OSE II was applied.

Photo above: Waveland Beach Mississippi June 15, 2011. This is the area where OSE II was applied on July 14th, 2010. OSE II was applied to the sandy beach on the north side of the concrete drain in order to compare the EPA allowed response with Corexit on the south side of the drain. OSE II cleaned the sandy beach completely, allowing the sand to remain free of oil. The boom protecting this demonstration area was recently removed. Go to this link http://osei.us/992 to see the video of the field application of OSE II at this Waveland Beach site. OSE II creates clean beaches and water and protects US natural resources.

Photo above: Waveland Beach Mississippi June 15, 2011: This is a closeup of the beach on the south side of the concrete drain, where OSE II was not applied, showing the effects of the EPA-allowed Corexit response. A large amount of oil is still present. Corexit destroys US Natural Resources.

Photo above: Waveland Beach, Mississippi, June 15, 2011. The swatch of dark-appearing grass is full of oil. OSE II was applied to the marsh grass and sand immediately to the right of that area and resulted in healthy grass and clean sandy beach.

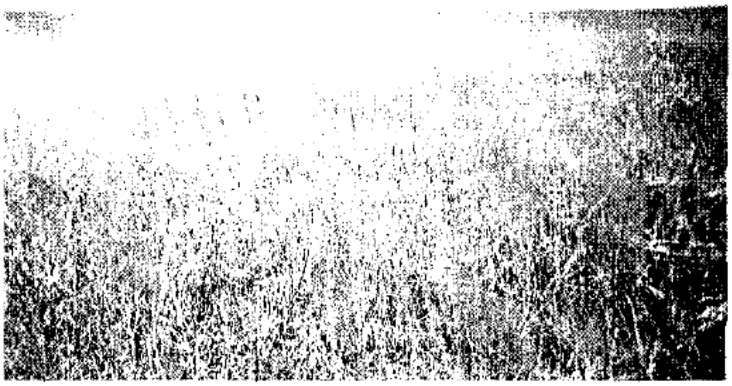

Photo above: Waveland Beach, Mississippi, June 15, 2011. This picture is just north west of the darkened adversely effected marsh in the previous picture above. The marsh grass is dying off from the EPA allowed response with Corexit. This picture shows deleterious effects of Corexit destroying natural resources and can be compared to the picture above where OSE II was applied to a small area of marsh grass just east of this spot, creating clean, protected US natural resources.

This demonstration absolutely proves there is no legitimate concern related to the use of OSE II and that it should be implemented immediately to begin to reverse the damage that has been done to the shorelines, estuaries, marshes, water column, sea floor, marine life and wildlife of the Gulf by the EPA's inadequate cleanup response to the Deepwater Horizon oil with both Corexits.

The fact is, with the Waveland Beach demonstration, an OSE II field demonstration has already been successfully performed and, predictably, with no adverse effects. The dichotomy between the proven constructive and valuable results of OSE II and the destructive impacts of the two Corexits clearly illustrate how the US EPA/NOAA, and other federal agencies on the RRT needlessly forced the Gulf Coast States of Louisiana, Mississippi, Alabama, Texas and Florida to suffer natural resource damages, unnecessarily exterminated millions of marine animals, pointlessly caused the destruction of thousands of birds, wreaked havoc on Gulf businesses, jobs and the economy, inflicted severe and alarming health problems and even death on massive numbers of Gulf Coast residents and cleanup responders who have begun the slow, painful trek to contracting numerous types of cancer, and ultimately their deaths, which is the second time responders have been given life sentences for helping out in an oil spill (the Valdez spill being the first notable time). All of this was absolutely unnecessary.

COMPARING OSE II AND TOXIC CHEMICAL DISPERSANTS

The EPA/NOAA, and the other federal agencies on the RRT that have arbitrarily thwarted the use of OSE II are now faced with the reality that a side-by-side comparison has been drawn between OSE II's results and the inadequate response of using Corexit. BP, a major oil company, has successfully tested OSE II on this massive spill, requested OSE II's implementation, and the EPA has continued to prevent its use with trumped up, baseless, non-scientific excuses. And this, while an

almost unimaginable amount of harm is being done to the natural resources of the US and health, safety and welfare of US citizens. The EPA/NOAA, and the other federal agency officials involved, are violating their oaths of office, their job descriptions, and their agency's charter requirements.

The EPA/NOAA/RRT VI has successful test experience with OSE II (EPA/NETAC testing), and successful utilization (Osage Indian Reservation on US navigable waters). The EPA learned, first hand, of 100's of clean ups performed on navigable waters by the US Navy in San Diego Bay over a 3½ year span, with dolphins and whales nearby. There were no adverse effects from the constant use of OSE II over this 3½ year period in San Diego Bay; no dead whales, dolphins, fish or wildlife. This is in direct contradiction to the destruction Corexit has caused in the Gulf with EPA's approved response action. When a product has as much use as OSE II has had in a confined bay area such as San Diego Bay, if it had anything in it that would cause adverse reactions to the environment it would have shown up, and dead species would have rolled up on the shore. This continued field experience proves that the trumped-up concerns of the EPA/NOAA and other federal agencies on the RRT's, are unfounded and baseless.

As explained above, EPA reps also witnessed the successful demonstra-tion of OSE II at Waveland Beach, Mississippi. Now, by putting up unscientific and arbitrary road blocks to a highly effective method of oil spill cleanup, they are proving they have a hidden agenda of some kind related to the Deepwater Horizon disaster which does not include cleaning up the ongoing BP spill. The significant spill of over 5,000 gallons of crude oil spilled by Texaco in Electra Texas, where OSE II addressed 100% of the spill, protected the entire eco system and resulted in no dead marine or wildlife, and returned the pond to pre spill conditions in 18 days. This, once again, verifies that the stated concerns and excuses claimed by these federal agencies to justify not using OSE II to handle this catastrophe are insincere and scientifically unreasonable.

OSE II has been used on thousands of spills in foreign countries in both fresh and salt water spills without a single negative impact. It has addressed these spills as a first and only response tool, effectively cleaning up the spilled oil without the carnage and economic losses attendant to the use of Corexit. It's long history of successful implementation is voluminous evidence, again, that the federal agencies' excuses to not use OSE II are baseless, and their negligence shows a complete lack of regard for the oaths of their office and responsibilities to the environment and the public.

In Summary:

1.The EPA has denied the requests for implementation of OSE II by three State Senators, 1 Governor and the City of Destin, FL.

2. The EPA and RRT federal agencies have stopped the use of OSE II with 4

scientifically baseless excuses:

a) "concerned that OSE II sinks oil" (scientifically baseless and easily refuted with sound science and an actual BP test);

b) "NOAA will not allow a product with a surfactant" (no scientifically-based reason and easily refuted with sound science and OSE II toxicity tests);

c) "EPA/NOAA are concerned OSE II may enhance too much indigenous bacteria", (scientifically baseless, and easily refuted with proof, sound science, tests, field use photos, and videos),

d) DOC (Department of Commerce) who has no scientific background with NOAA states they "will not allow a product with chemical surfactant", (easily refuted with sound science; OSE II toxicity tests on marine species; successful, safe field use for 16,000 spills; Waveland Beach, Mississippi demonstration; and human ingestion of OSE II).

3.The EPA/NOAA ignored the Coast Guard letter July 10,2010, which stated "take action with OSE II".

4. The EPA, without stating their reason, denied several requests by the LA DEQ to demonstrate or utilize OSE II after the DEQ had done extensive follow up vetting from May 5, 2011 and felt confident with moving forward with OSE II;

5. Sometime between May 19 and May 21, 2010, the EPA denied BP's request to use OSE II.

6. The EPA has denied BP's request to perform field trials with OSE II, despite the fact that OSE II showed, in tests conducted by BP in LSU labs, that it is, by far, the most effective product. They justified their decision by invoking a baseless, non-scientific reason (OSE II has a surfactant), a disingenuous and fabricated concern that can be easily dispelled by simply reviewing the numerous toxicity tests done on OSE II, all of which show that it is completely non-toxic.

7. A successful field demonstration of OSE II on Deepwater Horizon oil was performed at Waveland Beach, Mississippi on the sandy beach and in the marsh grass which proved, once again, that OSE II would effectively and swiftly clean up not only the oil but the toxic chemical dispersant, protecting the public's health, allowing the marine life and the flora and fauna to rehabilitate. This would allow the seafood and tourism industries to recover.

8. OSE II is extensively used as a first and only non-toxic response tool in other countries to swiftly and thoroughly return impacted areas to their pre-spill conditions with absolutely no negative downside or "trade offs." It has now cleaned up over 16,000 oil spills. This is in stark contrast to the use of chemical dispersants whose only function is to sink the oil beneath the surface and spread it broadly throughout the water column.

In light of all of the above, I, Steven R. Pedigo the individual, and the OSEI Corporation hereby request the immediate approval of the implementation of OSE II, and that a permit be issued for the use of OSE II on BP's Deepwater Horizon Macondo oil blowout in the waters of the Gulf of Mexico that began, per reports, on April 20, 2010.

Also, in light of all of the above, I, Steven R. Pedigo the individual, and the OSEI Corporation hereby request the immediate permanent pre-approval of OSE II for US navigable waters of Region VI.

Please send confirmation and/or the documents for both formal requests above as soon as possible.

Sincerely,
Steven Pedigo

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MARINE TOXICITY TEST SUMMARY
14 Toxicity Tests

OSEI Corporation, in its attempt to prove ***"Oil Spill Eater II"*** is virtually non-toxic, had the following tests performed:

The **MYSIDOPSIS BAHIA (or Mysid)** is one of the more sensitive marine organisms found in the oceans. LC50's (Lethal Concentration) is the level in which there is mortality with 50% of the species being tested. The lethal concentration calculated for OSEII on the Mysid was calculated once 10% of the test species showed equilibrium problems or mortality. At 96 hours, only 10% of the test species showed equilibrium problems or mortality at a calculated level of 2100 mg/L or 2,100 parts per million. This shows OSEII to have a low toxicity level, and had a true LC50 been performed the toxicity level would have been even lower.

The **MUMMICHOG (Fundulus Heteroclitus)** a somewhat larger organism (1 to 1.5 inches long) was tested to see how toxic OSEII was to it. 5,258 mg/L was established. 5,285 parts per million shows a very little toxicity for the Mummichog when exposed to Oil Spill Eater II.

MEDIAN LETHAL CONCENTRATIONS (LG50's) were calculated on Artemia Salina. The tests were run for 48 hours. OSEII alone tested greater than 100 mg/L so the true LG50 was not determined, but OSEII toxicity was greater than the EPA's cut-off for approving a product for the National Contingency Plan. There were other interesting facts involved with this toxicity test. The test calculation was based on using our product at a stronger concentration than our instructions allow. So at our instructed use rate, the toxicity level would have been even lower, even though the test was based on 100 mg/L or greater value. No. 2 fuel oil was tested alone and showed a level of 12.6 mg/L at 48 hours and No. 2 fuel oil and OSEII together at 48 hours showed a level of 29.4 helping

prove our point that once OSEII is applied, it immediately starts detoxifying hydrocarbons so bacteria can devour the hydrocarbons. (It is more beneficial to the environment to apply OSEII immediately, than to wait around for evaporation or to try to pick up the hydrocarbons mechanically.)98

OSEI Corporation feels the toxicity tests run in conjunction with OSEII help prove OSEII is virtually non-toxic. The EPA established that 35 mg/L LC50 was acceptable for a particular product to be used on the Exxon Valdez spill. If you compare OSEII to this established toxicity of 35 mg/L, then OSEII is far less toxic than that.
OSEI Corporation had two (2) fresh water toxicity tests run also. Environmental Canada, the U.S. EPA's equivalent in Canada, performed a toxicity test on rainbow trout. Rainbow trout are very sensitive fresh water species. The LC50 was greater than 10,000 mg/L. This shows OSEII to have virtually no toxicity in fresh water as well as salt water.
The other fresh water test was run on fathead minnows for the physical engineer in Plano, Texas, USA. We were attempting to prove that hydrocarbons which have had OSEII applied to them and then washed in the storm drain would not add any toxicity to the storm drain.
Two gallons of gasoline was poured onto a low area in a commercial business parking lot, and OSEII was applied, allowed to set 3 minutes, and then washed to another low area for collection.

Approximately 1 ••• gallons of runoff was collected and taken to the lab where a 48 hour fathead minnow survival test was initiated. The resulting LC50 test was 9,300 mg/L which shows that gasoline which has had OSEII applied to it is rendered virtually non-toxic.
This helped alleviate the physical engineer's concerns for adding anything toxic to the storm drain and ultimately to a creek, river or lake.
This test shows that using OSEII would help reduce the toxicity to storm drains from rain water runoff. If OSEII is used periodically to clean the parking lot allowing the site to stay within its NPDES permitted discharge levels.

Sincerely,
Steven Pedigo
Chairman

SP/eem99 OIL SPILL EATER INTERNATIONAL, CORP.

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SUMMARY
EPA/NETAC TOXICITY TEST
MYSIDOPSIS BAHIA

The Environmental Protection Agency in Gulf Breeze, Florida tested OIL SPILL EATER II Concentrate, for toxicity using a sensitive species named "Mysidopsis Bahia". This test was in conjunction with Efficacy Tests performed by the EPA and NETAC.

The LC50 for the acute (96 hr.) test was greater than 1,900 and up to 10,000 mg/L which shows OSE II to be virtually non-toxic.

The EPA allowed the use of Inipol during the Valdez Spill and Inipol's LC50 was 135 mg/L which would seem to OSEI, Corp to be somewhat toxic considering Environmental Canada's cut off is 1,000 mg/L.

A second LC50 was performed at 7 days to see if there was any problem with chronic toxicity. The LC50 was 2,500 mg/L, which once again shows OSE II to be virtually non-toxic even when the species was exposed in a closed environment for 7 days. It would be extremely difficult for a species to be exposed to OSE II for 7 days in an open system due to currents, wind and tidal actions.

This 3rd party, U.S. EPA Toxicity Test absolutely proves OSE II is virtually non-toxic.

By: Steven R. Pedigo
Chairman/OSEI, Corp.

SRP/AJL100

OIL SPILL RESPONSE BIOREMEDIATION AGENTS
EVALUATION METHODS VALIDATION TESTING
DISCUSSION OF RESULTS

The following data are provided for the oil spill response bioremediation agent producer as a means to begin to assess how this bioremediation agent may behave in response to an oil spill in the environment.
The Tier II 96-hour toxicity test data was conducted with Mysidopsis bahia test species. Mortality was the single measure response, therefore, survival data were used to calculate the 96-hour LC50. LC50 is the lowest concentration effecting 50% mortality of the test organism during a 96 hour exposure period. Sub-lethal and lethal responses were noted at concentrations between 1,000-10,000 mg/L (> 1,900 mg/L) following acute exposure of M.bahia to your bioremediation product.
Oil Spill Eater II was shown to cause a statistically significant reduction ($p = 0.05$) in the survival of Mysidopsis when animals were exposed during a chronic estimator test for a 7 day period. In general, 7 day exposure (2,500 mg/L) correlated well with values calculated following the 96 hour exposure (> 1,900 mg/L). NETAC101

TIER II TOXICITY DATA
TABLE 1

ACUTE TOXICITY VALUES FOR 96 HOUR LC_{50} – MYSIDOPSIS BAHIA

LC_{50} = Lethal concentration of product that will cause the death of 50% of the test species population within a defined exposure time.
a = LC50 presented as a range of test concentrations since data were from 96-hour acute range-finding test.
b = LC50 presented as a single, numerical value since data were from a definitive 96-hour acute toxicity test.
ND = Not Determined

TABLE 2

CHRONIC TOXICITY VALUES FOR 7 DAY LC_{50} – ***MYSIDOPSIS BAHIA***

NOEC = No Observable Effect Concentration
LOEC = Lowest Observable Effect Concentration
CI = Confidence Interval
NE = No Effect
Fecundity = Egg Production

As we indicated prior and to better understand the data presented above we are including a copy of the Evaluation Methods Manual. The Statistical Method Summary is found in Section 4, Method #8, page 40, of the manual and is intended to help a scientist understand the basis of the experimental objectives developed for this test.

Max. Test
Concentration
(mg/L)
Confidence
Interval
(95%)
96 hour LC50
(mg/L)
Product
1,000-10,000[a]
>1,900[b]
Oil Spill
Eater II
10,000
ND
7 Day LC50
(mg/L)
(95% CI)
Endpoints
(mg/L)
Effects
Measurement
Product

NOEC LOEC

5,700
NE
1,900
1,900
1,900
633
Survival
Growth
Fecundity
2,500(mg/L)
(2,225-3,313)

Oil Spill
Eater II NETAC102
Static Acute Toxicity of
Oil Spill Eater II, Batch 329,

To the Mysid, Mysidopsis bahia
Study Completed
March 9, 1990
Performing Laboratory
EnviroSystems Division

Resource Analysts, Incorporated
P.O. Box 778
One Lafayette Road
Hampton, New Hampshire 03842

I. SUMMARY

The acute toxicity of Oil Spill Eater II, batch 329 to the mysid, Mysidopsis bahia, is described in this report. The test was conducted for Incorporated for 96 hours during March 5-9, 1990 at the EnviroSystems Division of Resource Analysts, Inc. in Hampton, New Hampshire. It was conducted by Jeanne Magazu, Peter Kowalski, Robert Boeri, and Timothy Ward.

The test was performed under static conditions with five concentrations of test substance and a dilution water control at a mean temperature of 19.5°C. The dilution water was filtered natural seawater collected from the Atlantic Ocean at Hampton, New Hampshire. Aeration was not required to maintain dissolved oxygen concentrations above an acceptable level. Nominal concentrations of Oil Spill Eater II were: 0 mg/L (control), 1 mg/L, 10 mg/L, 100 mg/L, 1,000 mg/L, and 10,000 mg/L. Nominal concentrations were used for all calculations.

Mysids used in the test were less than 5 days old at the start of the test. They were produced at Resource Analysts, Inc. and acclimated under test conditions for their entire life. All mysids were in good condition at the beginning of the study.

Exposure of mysids to the test substance resulted in a 96 hour LC50 of 2,100 mg/L Oil Spill Eater II, with a 95 percent confidence level of 100 – 10,000 mg/L. The 96 hour no observed effect concentration is estimated to be 100 mg/L.

IV. METHODS AND MATERIALS

TEST SUBSTANCE:

Oil Spill Eater II (EnviroSystems Sample Number 2351E) was delivered to EnviroSystems on March 5, 1990. It was contained in a 500 ml plastic bottle that was labeled with the following information: Oil Spill Eater II, Batch 329. The sample was supplied by Incorporated. Prior to use the test material was stored at room temperature. Nominal concentrations were added to test media on a weight/vol basis and are reported as mg/L.

DILUTION WATER:

Water used for acclimation of test organisms and for all toxicity testing was seawater collected from the Atlantic Ocean at EnviroSystems in Hampton, New Hampshire. Water was adjusted to a salinity of 11-17 ppt (parts per thousand) and stored in 500-gallon polyethylene tanks, where it was aerated.

TEST ORGANISM:

Juvenile mysids employed as test organisms were from a single source and were identified using an approximate taxonomic key. They were produced and acclimated at the Resource Analysts, Inc. facility for their entire life. During acclimation mysids were not treated for disease and they were free of apparent sickness, injuries, and abnormalities at the beginning of the test. Mysids were fed newly hatched Artemia salina nauplii (EnviroSystems lot number

BS01) once or twice daily before the test.

TOXICITY TESTING:

The definitive toxicity test was performed during March 5-9, 1990. It was based on procedures of the U.S. Environmental Protection Agency (1986, 1987). The test was conducted at a target temperature of 20 ± 2°C with five concentrations of test substance and a dilution water control. A stock solution was prepared by combining 20.0 g of test substance with 2,000 ml of dilution water. The stock solution was added directly to dilution water contained in the test vessels without the use of a solvent. Nominal concentrations of the test material were: 0 mg/L, 10 mg/L, 100 mg/L, 1,000 mg/L, and 10,000 mg/L.

Twenty mysids were randomly distributed among a single replicate of each treatment. The test was performed in 2 liter glass dishes (approximately 25 cm in diameter and 8 cm deep) that contained 1.0 liter of test solution (water depth was approximately 4 cm). Test vessels were randomly arranged in an incubator during the 96 hour test. A 16 hour light and 8 hour dark photoperiod was automatically maintained with cool-white fluorescent lights that provided a light intensity of 40 eEs-1m-2. Aeration was not required to maintain dissolved oxygen concentrations above acceptable levels. Mysids were fed newly hatched Artemia salina nauplii once per day during the test.

The number of surviving organisms and the occurrence of sublethal effects (loss of equilibrium, erratic swimming, loss of reflex, excitability, discoloration, or change in behavior) were determined visually and recorded initially and after 24, 48, 72, and 96 hours. Dead test organisms were removed when first observed. Dissolved oxygen (YSI Model 57 meter; instrument number PRL-3), pH (Beckman model pHI 12 meter; instrument number PRL-4), salinity (Labcomp SCT meter, instrument number PRL-6), and temperature (ASTM mercury thermometer; thermometer number 2211) were measured and recorded daily in each test chamber that contained live animals.

STATISTICAL METHODS:

Results of the toxicity test were interpreted by standard statistical techniques. Computer methods (Stephan, 1983) were used to calculate the 96 hour median lethal concentration (LC50). The no observed effect level is the highest tested concentration at which 90% or more of the exposed organisms were unaffected.

V. RESULTS

No insoluble material was observed in any test vessel during the test. Biological and water quality data generated by the acute toxicity test are presented in Table 1 and Appendix A, respectively. One hundred percent survival occurred in the control exposure.

The dose – response curve for organisms exposed to the test substance for 96 hours is presented in Figure 1. Exposure of mysids to the Oil Spill Eater II, batch 329, resulted in a 96 hour LC50 of 2,100 mg/L, with a 95 percent confidence interval of 100 – 10,000 mg/L. The 96 hour no observed effect concentration is estimated to be 100 mg/L.

Table 1. Survival data from toxicity test

Nominal Concentration	Number Alive					Number Affected				
(mg/L)	0hr	24hr	48hr	72hr	96hr	0hr	24hr	48hr	72hr	96hr
0 (control)	1	10	10	10	10	0	0	0	0	0
1	1	10	10	9	9	0	0	0	0	0
10	1	10	10	9	9	0	0	0	0	0
100	1	10	10	10	9	0	0	0	0	0
1,000	1	10	9	9	8	0	0	0	0	0
10,000	1	10	0	0	0	0	0	-	-	-

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TOXICITY TEST FOR ARTEMIA SALINA

To gain acceptance on the U.S. EPA's National Contingency Plan List, we were requested to perform an additional Toxicity Test on Artemia Salina using EPA's Standard Dispersant Toxicity Test.

OSE II Concentrate was presented to the laboratory, but the laboratory refers to the product as a Dispersant instead of OSE II throughout the write-up, since it was a Dispersant Toxicity Test. The Test proved that OSE II Concentrate is once again virtually non-toxic. This particular test proved OSE II helps to detoxify oil. The fuel oil had a higher toxicity rate than did the fuel and OSE II, which shows OSE II to immediately starts reducing the toxicity of hydrocarbons once OSE II is applied. The fuel oils toxicity was 12.4 ppm, and the fuel oil and with OSE II applied showed a drop in the fuel oils toxicity to 29.4, over a 100 percent reduction of the toxicity of the fuel oil. This shows real value in utilizing OSE II since the toxicity of the spilled contaminant would be reduced immediately lesoning the impact of a spill to the associated environment and marine species.

OSE II gained acceptance to the EPA's National Contingency Plan once this test was presented to the EPA.

By: Steven R. Pedigo
Chairman, OSEI, Corp.

SRP/AJL111

Standard Dispersant Toxicity Test with the
OSE II, Batch #9820 and Artemia salina

Authors
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Hampton, New Hampshire 03842
October, 1990

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IV. INTRODUCTION

The objective of the study was to determine the acute toxicity of the dispersant – Batch # 9820, No. 2 fuel oil, and a 1:10 mixture of dispersant and oil to *Artemia salina*, a marine invertebrate. The report contains sections that describe the methods and materials employed in the study, and the results of the investigation. The report also contains an appendix that presents the water quality data collected during the tests.

V. METHODS AND MATERIALS

TEST SUBSTANCE:

The dispersant – Batch # 9820 (EnviroSystems Sample Number 2591E) was delivered to EnviroSystems on August 17, 1990. It was contained in two 1,000 ml plastic bottles that were labeled with the following information: "Batch # 9820". The No. 2 fuel oil (EnviroSystems Sample Number 2599E) was delivered to EnviroSystems on August 28, 1990. It was contained in a 1,000 ml plastic bottle that was labeled with the following information: "# 2 fuel oil".

DILUTION WATER:

Water used for hatching and acclimation of test organisms and for all toxicity testing was formulated at EnviroSystems in Hampton, New Hampshire. Water was diluted to a salinity of 20 parts per thousand and stored in polyethylene tanks where it was aerated.

TEST ORGANISM:

Juvenile *Artemia salina* employed as test organisms were from a single source and were identified using an appropriate taxonomic key. *Artemia salina* used in the test were produced from an in-house culture and were 24 hours old at the start of the test. Prior to testing, *Artemia salina* were maintained in 100% dilution water under static conditions. During acclimation *Artemia salina* were not treated for disease and they were free of apparent sickness, injuries, and abnormalities at the beginning of the test. They were not fed before or during the tests.

TOXICITY TESTING:

Screening tests with the test substances were conducted during October 1 to 3, 1990. The definitive toxicity tests were performed with the dispersant, No. 2 fuel oil, a 1:10 mixture of dispersant and oil, and the standard toxicant, dodecyl sodium sulfate during October 3 to 5, 1990, according to procedures of the U.S. EPA (1984). The tests were conducted at a target temperature of 20 ± 1°C with five concentrations of each test substance and a dilution water control.

The dispersant and oil stock solutions were prepared by combining 550 ml of sea water and 0.55 ml of test substance in a glass blender jar and mixing the solution at 10,000 rpm for 5 seconds. The combined dispersant and oil stock solution was prepared by mixing 550 ml of sea water at 10,000 rpm and adding 0.5 ml of oil and 0.05 ml of dispersant. This combined mixture was then mixed for 5 seconds. Nominal concentrations of each test material were: 0 mg/L (control), 10 mg/L, 25 mg/L, 40 mg/L, 60 mg/L, and 100 mg/L. Media in each test vessel was added at the beginning of the test and not renewed.

Twenty *Artemia salina* were randomly distributed to each of 5 replicates of each treatment. The tests were performed in 250 ml glass Carolina culture dishes that contained 100 ml of test solution (water depth was approximately 2.5 cm). Test vessels were randomly arranged in an incubator during the 48 hour test. A 24 hour light and 0 hour dark photoperiod was maintained below the dishes. Aeration was not required to maintain dissolved oxygen concentrations above acceptable levels. *Artemia salina* were not fed during the tests.

The number of surviving organisms was determined visually and recorded initially and after 24 and 48 hours. Dead test organisms were removed when first observed. Dissolved oxygen (YSI Model 57 meter; instrument number PRL-18), pH (Beckman model pHI 12 meter; instrument number PRL-4), salinity (Refractometer, instrument number PRL-6), and temperature (ASTM mercury thermometer; thermometer number 2211) were measured and recorded at the beginning and end of each test in one test chamber of each concentration.

STATISTICAL METHODS:

Results of the toxicity test were interpreted by standard statistical techniques (Stephen, 1983). The binomial method was used to calculate the median lethal concentration (LC50) values.

VI. RESULTS

All test vessels containing dispersant appeared clear throughout the test and all test vessels containing oil or oil and dispersant had an oil slick on the surface of the test media throughout the test. Biological and water quality data generated by the acute toxicity tests are presented in Table 1 and Appendix A, respectively. Ninety-nine percent survival occurred in the control exposure. The 48 hour LC50 for *Artemia salina* exposed to the reference toxicant dodecyl sodium sulfate is 38.7 mg/L.

The 24 and 48 hour LD50s from the three toxicity tests are presented in Table 2. The 48 hour LC50s for *Artemia salina* exposed to the test substances are: dispersant/OSE II - >100 mg/L, No. fuel oil – 12.6 mg/L (95% confidence interval = 10.0 – 25.0 mg/L), and a 1:10 mixture of dispersant/OSE II and
No. 2 fuel oil – 29.4 mg/L (95% confidence interval = 25.0 – 40.0 mg/L).

Table 1. Survival data from toxicity tests

Number Alive									
Nominal Concentration (mg/L)	Dispersant/OSE II rep.	0hr	24hr	48hr	No. 2 fuel oil 0hr	24hr	48hr	Oil + Dispersant/OSE II 0hr	24hr	48hr
0 (control)	1	20	20	20	20	20	20	20	20	20
	2	20	20	19	20	20	19	20	20	20
	3	20	20	20	20	20	20	20	20	20
	4	20	20	20	20	20	20	20	20	20
	5	20	20	20	20	20	20	20	20	20
10	1	20	19	17	20	20	17	20	20	19
	2	20	20	17	20	20	19	20	20	18
	3	20	20	20	20	20	12	20	18	18
	4	20	20	19	20	20	9	20	20	17
	5	20	19	18	20	18	10	20	20	16
25	1	20	20	16	20	18	0	20	19	19
	2	20	19	17	20	19	3	20	18	15
	3	20	20	18	20	19	2	20	20	16
	4	20	19	12	20	20	2	20	20	17
	5	20	19	15	20	20	0	20	19	14
40	1	20	19	16	20	20	0	20	19	0
	2	20	20	14	20	19	0	20	20	0
	3	20	20	19	20	20	0	20	20	0

4 20 20 15 20 18 0 20 14 0
5 20 20 17 20 17 0 20 18 2
60 1 20 19 18 20 18 0 20 18 0
2 20 19 16 20 19 0 20 19 0
3 20 19 19 20 16 0 20 19 0
4 20 20 17 20 19 0 20 16 0
5 20 20 16 20 14 1 20 16 1
100 1 20 20 18 20 13 0 20 20 0
2 20 20 18 20 8 0 20 20 0
3 20 19 13 20 9 0 20 20 0
4 20 20 19 20 10 0 20 20 0
5 20 20 16 20 8 0 20 20 0

VII. REFERENCES

Stephen, C.E. 1983. Computer program for calculation of LC50 values. Personal communication.
U.S. EPA. 1984. Revised Standard Dispersant Toxicity Test. Federal Register, Volume 49, Number 139, Wednesday, July 18, 1984, pages 29204 to 29207.

Appendix A. WATER QUALITY DATA FROM TOXICITY TESTS

I. Summary

The acute toxicity of the dispersant – Batch #9820, No. 2 fuel oil, and a 1:10 mixture of dispersant/OSE II and No. 2 fuel oil to *Artemia salina*, is described in this report. The test was conducted for OSEI corp for 48 hours during October 3 to 5, 1990, at the EnviroSystems Division of Resource Analysts, Inc. in Hampton, New Hampshire.

The test was performed under static conditions with five concentrations of each test substance and a dilution water control at a temperature of 20 $\pm$ 1°C. The dilution water was sea water adjusted to a salinity of 20 parts per thousand. Aeration was not employed to maintain dissolved oxygen concentrations above an acceptable level. Nominal concentrations of all three test substances were: 0 mg/L (control), 10 mg/L, 25 mg/L, 40 mg/L, 60 mg/L and 100 mg/L. Nominal concentrations were used for all calculations.

Artemia salina used in the test were 24 hours old at the start of the test and they were all in good condition at the beginning of the study. Exposure of *Artemia salina* to the test substances resulted in the following 48 hours median lethal concentrations (LC50): dispersant/OSE II >100 mg/L, No. 2 fuel oil – 12.6 mg/L (95% confidence interval = 10.0- 25.0 mg/L), and a 1:10 mixture of dispersant/OSE II and No. 2 fuel oil-29.4 mg/L (95% confidence interval = 25.0 – 40.0 mg/L).

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SUMMARY
ENVIRONMENT CANADA'S TOXICITY TEST

Environmental Canada performs Toxicity Testing for determining if a product could gain approval for use in Canada. The level that is considered toxic is 1,000 mg/L or less. A product that exceeds this level is deemed acceptable. The higher the number the less toxic.

Oil Spill Eater II Concentrate, tested at 10,000 mg/L – which shows OSE II Concentrate is virtually non-toxic and far exceeds the level deemed to toxic by Environment Canada.

Rainbow Trout is one of the most sensitive fresh water organisms to test. OSE II proved that even with third party testing by a Foreign Government, OSE II is virtually non-toxic.

By: Steven R. Pedigo
Chairman/OSEI, Corp.121

Environment Canada
Conservation and P ote tion
Emergencies Science Division
River Road Environmental Technology Centre
3439 River Road
Ottawa, Ontario K1A 0H3
May 17, 1993 4808-13-7
Steven R. Pedigo, Chairman,
OSEI Corporation
5545 Harvest Hill
Suite 1116
Dallas, TX 75230
U.S. A.
Dear Mr. Pedigo,

Thank-you for participating in the development of Environment Canada's draft guidelines for assessing the toxicity and effectiveness of oil spill bioremediation agents (OSBAs).

The Tier I toxicity testing is now complete. Our preliminary screening has indicated that the *Daphnia magna* test and the Microtox test were either insensitive or erratic. Therefore, we do not consider these particular tests useful for OSBA evaluation. Comments on the toxicity of your product will thus be limited to those obtained using the 96-hour Rainbow Trout acute lethality test. 'Oil Spill Eater II' had a rainbow trout 96-hour LC50 of greater than 10,000 mg of application solution per litre of water. There was, however, a 23% mean fish mortality at this concentration. Also note that between 24 and 96 hours of exposure to the product, sublethal effects were present. The fish were noted to surface, be on their side, turn dark, exhibit rapid breathing and no swimming. These sublethal effects should be of concern. The effectiveness test analyses are still being performed. You will be notified as soon as those results are available.

If your product meets both the effectiveness and toxicity criteria it will be placed on our Standard List of Oil Spill Bioremediation Agents. Placement on this list is not an indication that the product will be used in the event of an oil spill. The list and test results are public information. They may be provided to oil spill response personnel to enable them to make informed decisions.

Please take note that the placement of a product on our Standard List does not constitute an approval or certification or licensing of your product for use in Canada. Your product may be required to comply with the New Substances Notification Regulations (NSNR) for biotechnology products under the Canadian Environmental Protection Act (CEPA). For information on the draft regulations, please contact the Chief of the New Substances Division at (819) 997-4336 or at the following address: Chief, New Substances Division, CCB, Environmental Canada, P.V.M. 14th Floor, Ottawa, Ontario, K1A 0H3, CANADA.

Sincerely,
Merv Fingas
Chief, Emergencies Science Division

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ENVIRONMENT CANADA
TIER I TOXICITY TESTING
FOR EVALUATION OF DRAFT OSBA GUIDELINES

The testing was performed as follows. An application solution of the OSBA was prepared based on instructions provided by the manufacturer/supplier. The highest strength of solution tested was 10,000 mg of application solution per litre of water (approx. a 1:100 dilution). For products in which solids are normally added to the water, suspensions comprised of 10,000 mg of product/combined product per litre of water were prepared for use in the toxicity tests. (If several solids were to be added, they were combined in the appropriate ratio). This initial screening concentration was tested in triplicate. If this concentration was toxic to greater than 50% of the organisms, lower concentrations were tested. Sub-lethal effects on the behavior and/or appearance of the organisms were also made. The toxicity of the product in water was assessed using each of the following three biological test methods, developed and standardized by Environment Canada for these and other applications:

Environment Canada, 1990a. **Biological test method: acute lethality test using rainbow trout.** Environment Canada, Conservation and Protection, Ottawa, Ontario. Report EPS 1/RM/9, 51 pp.

Environment Canada, 1990b. **Biological test method: acute lethality test using *Daphnia* spp.** Environment Canada, Conservation and Protection, Ottawa, Ontario. Report EPS 1/RM/11, 57 pp.

Environment Canada, 1992. **Biological Test method: toxicity test using luminescent bacteria (*Photobacterium phosphoreum*).** Environment Canada, Conservation and Protection, Ottawa, Ontario. Report EPS 1/RM/24, 61 pp.

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TOXICITY TEST SUMMARY USINGCITGO GASOLINE, OIL SPILL EATER II AND FATHEAD MINNOWS

To prove OIL SPILL EATER II rapidly detoxifies hydrocarbons once OSE II is applied, a Toxicity Test was set up with the Physical Engineer of the City of Plano, Texas.

One half gallon of gasoline was poured onto a concrete surface, where ••• gallon of OSE II (pre-diluted 100 to 1 was immediately applied. The treated gasoline was allowed to set for two (2) minutes at which time two (2) gallons of fresh water were used to wash this effluent into a catch basin. Approximately 1 ••• gallons were recovered and sent to Bio-Aquatic Laboratory.

Bio-Aquatic Laboratory performed a Static 48 Definitive Toxicity Test using Fathead Minnows (*Pimphales promeas*). The LC50 was 9,300 mg/L which is a relatively low toxicity level.

This test shows that OSE II when applied to a toxic constituent rapidly reduces toxicity. This detoxifying action of OSE II limits the toxicity of a spill to marine organisms, and will allow Mother Nature's Bacteria to rapidly attack this detoxified spill. The rapid detoxification of a spill shows that OSE II is a beneficial tool for first response cleanup for a spill. This test also shows that if OSE II is used to clean up a parking lot and washed into the storm drain there would be no adverse environmental impact.

By: Steven R. Pedigo
Chairman/OSEI, Corp.124

OSEI CORPORATION
OSE II/GASOLINE/WATER
Toxicity Test Report
DECEMBER 7, 1991
BIO-AQUATIC TESTING, INC.

Prepared by: _____
David Smith,
Aquatic Toxicologist125
BIO-AQUATIC TESTING, INC.
1555 Valwood Parkway, Ste. 100
Carrollton, Texas 75006
Tel: (214) 247-5928
Fax: (214) 241-4474

TOXICITY TEST REPORT – ACUTE

Client . . . . . .OSEI Corporation Laboratory I.D. BO-12-91-2239
Sample . . . . . OSE II/Gasoline/Water Date . . . . . December 7, 1991
Results: The 48-hour LC50 for *Pimephales promelas* exposed to a mixture of OSE II, gasoline, and water was 9,300 mg/L.

SAMPLE COLLECTION

CHEMICAL MEASUREMENTS

TEST PROCEDURES

Pimephales promelas

Approximately one and a half gallons of runoff grab sample from an OSEI Corporation product demonstration was delivered to Bio-Aquatic Testing on December 5, 1991. The sample was manually collected by OSEI personnel. One toxicity test was requested: a static 48-hour definitive toxicity test using the fathead minnow (*Pimephales promelas*).

The sample was analyzed for residual chlorine (EPA Method 330.1, Amperometric Titration Method) and was determined to contain <0.10 mg/L. Sample and laboratory dilution water pH, temperature, conductivity, hardness, alkalinity and D.O. were analyzed and recorded daily.

The 48-hour fathead minnow larval survival test was initiated at 1450 hours, December 6, 1991. Five concentrations were established for testing (200 mg/L, 800 mg/L, 3,000 mg/L, 9,000 mg/L, and 30,000 mg/L) utilizing reconstituted distilled, deionized water as dilution water. The test was set up using distilled water rinsed 500 mL plastic cups as test chambers. Four replicate cups containing five organisms each in 250 mL of test solution were used per dilution. All organisms used were laboratory reared and less than 24 hours old at test initiation. The test was allowed to proceed for 48 hours during which mortality was recorded daily.

A control of four replicate chambers containing five organisms each in 100% synthetic laboratory water was conducted concurrently with the test. There was 100% survival in the control. Data on surviving organisms as well as water quality measurements were recorded on the data sheet. The test ended at 1450 hours, December 8, 1991. The acute toxicity data analysis program provided by the EPA was employed to determine the LC50 values.126

LC50 RESULTS

Pimephales promelas

SUMMARY

LC50 value calculated using the Binomial Method:

CONC. (mg/L)	# EXPOSED	# DEAD	% DEAD	BINOMIAL %
30,000	20	20	100	0.0001
9,000	20	6	30	5.7659
3,000	20	1	5	0.0020
800	20	0	0	0.0001
200	20	0	0	0.0001

The Binomial Test shows that 3,000 and 30,000 can be used as statistically sound conservative 95 percent confidence limits since the actual confidence level associated with these limits is 99.99791 percent.

An approximate LC50 for this set of data is 11,800 mg/L.

LC50 value calculated using the Trimmed Spearman-Karber Method:

Trim	Var. of Ln Est.	LC50	95% Conf. Limits
0.00%	0.17396D-01	9,300 mg/L	7,100 to 12,100 mg/L

The 48-hour LC50 for *Pimephales promelas* exposed to a mixture of OSE II, gasoline, and water was 9,300 mg/L.

BIO-AQUATIC TESTING, INC.

48 – HOUR *PIMEPHALES PROMELAS* ACUTE TOXICITY TEST

CLIENT OSEI Corporation BEGIN DATE 12/06/91
SAMPLE OSE II, Gasoline, Water END DATE 12/08/91
LAB ID **# BO-12-91-2239B** TEST ORGANISM *Pimephales promelas*
DATE COLLECTED 12/05/91 TEST TEMPERATURE (°C) 25° ± 1
DATE RECEIVED 12/05/91 PHOTO PERIOD 16 hour light / 8 hour dark
SAMPLE TYPE Grab LIGHT INTENSITY 75 FT-C
TEST TYPE Acute ANALYST W. Smith

EFFLUENT MEASUREMENTS

D.O. @ 30,000 mg/L, 8.6/6.6
pH @ 30,000, 8.3/8.4
CONDUCTIVITY @ 30,000 (µMHOS) 500
HARDNESS (mg/L as CaCO3) 272.4 ALKALINITY (mg/L as CaCO3) 625.0

DECHLORINATION

RESIDUAL Cl_2 (mg/L) <0.10 ANALYSIS METHOD Amperometric Titration Method (330.1)
DECHLORINATION REAGENT Not Applicable

DILUTION WATER MEASUREMENTS

D.O. @ 100% (mg/L), 8.6/6.9
pH @ 100%1 8.4/8.3
RECEIVING WATER DILUTION WATER Laboratory adjusted
HARDNESS (mg/L as CaCO3) 160.0 ALKALINITY (mg/L as CaCO3) 107.0
Recorded at the beginning and end of each 24-hour exposure period.

SURVIVAL SUMMARY

x % Surv.	x LIVE PER CONC
	100
	100
	100
	95
	70
	0
	% EFFLUENT

CONC	NUMBER LIVE PER REP											
	START				24 HOURS				48 HOURS			
	a	b	c	d	a	b	c	d	a	b	c	d
Control	5	5	5	5	5	5	5	5	5	5	5	5
200 mg/L	5	5	5	5	5	5	5	5	5	5	5	5
800 mg/L	5	5	5	5	5	5	5	5	5	5	5	5
3,000 mg/L	5	5	5	5	5	5	5	5	5	5	4	5
9,000 mg/L	5	5	5	5	3	3	5	5	3	1	5	5
30,000 mg/L	5	5	5	5	0	0	0	0	0	0	0	0

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EPA in Cooperation with NETAC a Group out of

Pittsburgh University performed Efficacy and ToxicityTesting

on OSE II for the EPA NCP Protocol Development.

The Summary follows

The OSEI Corporation supplied OSE II to Hap Prichard of the US EPA in 1992. The EPA performed two separate tests a 48 hour exposure test and a 96 hour exposure test, on two different species Mysidopsis Bahia, and Menidia beryllina. The Mysidopsis Bahia tests also contained a static renewal LC50 for 48 hours and 96 hours with OSE II, and a 7 day toxicity test as well.

The test information is contained in the five pages following this summary, as well as the freedom of information request that was honored over five (5) years after it was requested for these tests shows the ODSEI Corporation received this information from the US EPA. The test information with the redacted black outs, is as the OSEI Corporation received them, from the US EPA.

Toxicity tests are performed to show the potential effects of a product to marine species. The larger or higher the number the less toxic the product is. LC 50, the LC means lethal concentration, or the concentration of a product to produce death of the test species.

The US EPA's first toxicity test of OSE II was on Mysidopsis Bahia for 48 hours of exposure, and for 96 hours of exposure. The 48 hour exposure toxicity test showed OSE II's toxicity value to be between 5,661 to 7,927 for an average of 6,698. The 96 hour exposure toxicity test showed OSE II's toxicity value to be between 3,125 to 6,250 for an LC 50

of 5,970. These two test shows the US EPA has proven OSE II to be virtually non toxic.

The US EPA static renewal LC 50 with OSE II and the Mysidopsis Bahia was >5,700 for the 48 hour exposure, and >5,700 as well. The EPA established values for OSE II with this species for both exposure times proves OSE II is virtually non toxic.

The US EPA went on to perform a seven (7) day toxicity test with OSE II and the Mysidopsis Bahia. The LC 50 was 2,225 to 3,133, for an LC 50 value of 2,500 which for a seven (7) day toxicity test is phenomenally non toxic.

The US EPA performed toxicity tests on a second species for the EPA/NETAC testing Menidia beryllina. The first test on this species was for an exposure time of 48 hours, and the LC 50 value was 6,250 to 12,500 for an LC 50 value of 8,839. The second test with the Menidia beryllina was for the exposure time of 96 hours, and the value was between 6,250 and 12,500 as well for an LC 50 of 8,839. These two test show the US EPA proving OSE II is virtually non toxic on a second species

These toxicity tests associated with the US EPA/NETAC testing as well as the numerous other toxicity tests that have been performed with OSE II by the US EPA and other governments, and for other governments by the OSEI Corporation overwhelmingly prove OSE II is safe for any marine environments species. These toxicity tests show that when OSE II is utilized for a spill there is real value obtained by using OSE II since it converts a spill to CO 2 and water while limiting and or reducing the toxicity of the spill to the environment.

Steven Pedigo

OSEI Corporation

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
NATIONAL HEALTH AND ENVIRONMENTAL EFFECTS
RESEARCH LABORATORY
RESEARCH TRIANGLE PARK, NC 27711

June 25, 2003

OFFICE OF
RESEARCH AND DEVELOPMENT

Mr. George Lively
Oil Spill Eater International Corp.
13127 Chandler Drive
Dallas, Texas 75243

RECEIVED
BY ORL DATE 6-30-03

re: Freedom of Information Act Request HQ-RIN-01971-02

Dear Mr. Lively:

In response to your request for records under the Freedom of Information Act, we were asked to search for and provide data generated using Product C at the Gulf Ecology Division (GED) during the development of oil spill bioremediation protocols. The research involved several laboratories, both within the Office of Research and Development and outside of the Agency.

We are providing these data as an enclosure to this letter, at no cost to you. We also offer a quick explanation of these data in the hopes that it will facilitate your understanding and use .

It is important to note that we used a variety of commercial bioremediation products (CBAs) to develop and evaluate test systems and protocols for the purpose of assessing the efficacy and environmental safety (toxicity) of current and future oil spill bioremediation agents; thus, any data generated with a particular (CBA) was not primarily for the intent of evaluating the product but rather for the purpose of evaluating the test systems under development. These CBAs were provided to us, blind coded, by NETAC--at no time during the collection of these data did we know the actual name of the vendor or product, and thus none of the data will have a vendor's name or product identification associated with it.

In our data, we sometimes refer to Product C as Product 1 - 3 or as CBA C; we have also referred to it by another letter (see manuscript information, below). Data generated at GED was developed through collaborative studies (two cooperative agreements) with the University of West Florida. Throughout the course of evaluating the tests systems, data from more than one CBA might be discussed in notebooks on the same day. Where we have included copies of this data, we have crossed through information that does not respond to FOIA Request HQ RIN-01971-02.

In order to put the data provided in its proper perspective, a copy of a publication and parts of a manuscript are provided to serve as entry points to understanding the data, logs, and materials in this package.

Protocol development utilized a tiered approach of increasingly complex test systems for product evaluation, which is described in more detail in the EPA publication EPA/600/X-93/001 (mentioned below). There were three primary aspects of this research which were conducted at GED that generated data with CBA C:

1. Development of a Tier II Environmental Safety Protocol which focused on the intrinsic toxicity of the bioremediation agent alone and in conjunction with a water soluble oil fraction.

   A manuscript entitled "Evaluation of Protocols to Assess Efficacy and Environmental Safety of Commercial Oil Spill Bioremediation Agents: Agent Toxicity" addresses the Tier II Environmental Safety Protocol; excerpts from this manuscript which include data on Product C are provided. It is important to note that, due to lack of data on all ten products, the products were re-labeled, and **Product C appears in this manuscript as Product "B".** Final editing following review has not been completed, and thus **we request that information in the manuscript not be quoted or cited.** Toxicity data generated at GED that we are providing on this research component includes:

   Menidia beryllina 96-h Static Test with Product C (CBA C)

   Range Finding Acute Test with *Mysidopsis bahia* Using Product C (1 - 3)

   96-h Static Acute Test with 7-day *Mysidopsis bahia* Using Product C (CBA C). [This test was rejected due to low dissolved oxygen concentrations.]

   96-h Static Acute Test with 7-day *Mysidopsis bahia* Using Product C (CBA C)

2. Development of the Tier III Simulated Open Water Test System, which examined the efficacy of a bioremediation agent using a simulated open water/oil slick system.

   The following publication contains a description of Tier III testing as well as summaries of Tier III efficacy data with Product C:

   Lepo, Joe Eugene. 1993. Evaluation of Tier III Bioremediation Agent Screening Protocol for Open Water Using Commercial Agents: Preliminary Report. EPA/600/X-93/001. U.S. Environmental Protection Agency, Environmental Research Laboratory, Gulf Breeze, FL. 27 p.

   Microbiological data supporting this research is identified as:

   Microbiology Associated with Tier III Efficacy Test of Product 1 - 3 (Data from Two Notebooks)

   Analytical Chemistry data for this component is provided as:

   Extraction and Preparation of Samples from Tier III Efficacy Test of Product 1 - 3, Including GC Analysis, and Preparation for GC/MS Analysis

   Gravimetric Data for Tier III Efficacy Test of Product 1 - 3

   Gravimetric/Effluent Data from Tier III Efficacy Test of Product 1 - 3

GC/FID Data for Tier III Efficacy Test of Product 1 - 3

Daily Log of GC/MS Samples

GC/FID Data from Tier III Efficacy Test of Product 1 - 3

GC/MS Data for Tier III Efficacy Test of Product 1 - 3

3. Development of the Tier III Open Water Toxicity Test, which evaluated the toxicity of effluent generated by the Tier III Open Water Test System.

EPA publication EPA/600/X-93/001 (mentioned above) contains summaries of Tier III toxicity data with Product C. Toxicological data supporting this research includes:

7-day Chronic Estimator Test with *Mysidopsis bahia* Using Effluent from Tier III Microcosms with Product C (1 - 3)

We hope you find this explanation, description and records helpful.

Enclosures

Sincerely,

John C. Jones
Deputy Director for Management
National Health and Environmental
Effects Research Laboratory

TOXICOLOGY

NOTEBOOK: 984

PAGES: 1 - 4

MENIDIA BERYLLINA 96-H STATIC TEST WITH PRODUCT C (CBA C)

Table 5. 48, 96 h, and 7-d LC50 values (95% confi. lim.)[a] for CBAs in static and static-renewal tests using *M. beryllina* and *M. bahia*.

	static LC50		static-renewal LC50		
CBA	48-h	96-h	48-h	96-h	7-d
Mysidopsis bahia					
B	6,698 (5,661-7,927)	5,970 (5,125-6,250)	>5,700	>5,700	2,500 (2,225-3,133)
Menidia beryllina					
B	8,839 (6,250-12,500)	8,839 (6,250-12,500)	---		

[a]Nominal concentrations (mg/L).
[b]Short-term chronic test not conducted.

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Date June 30, 2008

Fresh Water Marine Toxicity Test Summary

South Korea (Minnows)

The OSEI Corporation performed a toxicity test for the Korean Government approval process involving minnows (Pimephales promelas). The toxicity test was a 24 hour acute toxicity test. The LC50 value for this test was 707.11 mg/l at a 20% concentration, which is the concentration the Korean government test required. If you extrapolate the test value, had the test been performed at the OSE II application concentration of 2% instead of 20%, then the LC50 would have been over 1337.11 mg/l which proves OSE II to be virtually non toxic. There are several government agencies around the world that try to force specific tests to be performed at a single concentration without allowing for the application rate of a product. So while they come up with a value at a certain concentration it may, or may not be applicable to every product, which is why we point out the extrapolation calculation for OSE II at the recommended application rate.

Steven Pedigo

Chairman/CEO OSEI Corporation

OIL SPILL EATER II (2%)
ACUTE PRODUCT TEST

June 2008

24-Hour Acute Toxicity Test Results

Pimephales promelas

Prepared for:

Kwang Keun, Kim
Korea Institute of Construction anticorrosive Technology
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TABLE OF CONTENTS

ACUTE LC50 PRODUCT REPORT

Client . . . . . OSEI, Corporation
Sample . . . . . .Oil Spill Eater II
Project No. OS457
Test Date . . . . . June 2008

Results:

24-hr. *P. Promelas* LC50: 5,856.34 mg/L
95% Upper Confidence Limits: 6,265.67 mg/L
95% Lower Confidence Limits: 5,473.76 mg/L

INTRODUCTION

A product identified as Oil Spill Eater II, Concentrate was delivered to Huther and Associates, Inc. on June 26, 2008. One acute toxicity test was conducted: a static acute 24-hour definitive toxicity test using *Pimephales promelas* (fathead minnow). Test procedures followed recommended methods contained in "*Methods for Measuring the Acute Toxicity of Effluents and Receiving Waters to Freshwater and Marine Organisms, Fifth Edition*", EPA-821-R-02-012, October 2004.

P. promelas are a freshwater aquatic indicator organism frequently used to evaluate the potential toxicity of a compound or an effluent. The acute toxicity of a compound or effluent is generally measured using a multi-concentration, or definitive test, consisting of a control water and a minimum of five increasing concentrations of product added to control water. The test is designed to provide dose-response information, expressed as the concentration that is lethal to 50% of the test organisms (LC50).

SAMPLE PREPARATION

Oil Spill Eater II was initially prepared for definitive testing by adding the product to distilled, deionized water at a ratio of 50 parts water to 1 part product (2% concentration; stock solution). Seven test concentrations of stock solution were prepared in distilled, deionized water reconstituted to 104 mg/L as $CaCO_3$. The seven concentrations were 250, 500, 1000, 2000, 4000, 8000 and 16,000 mg/L. Dissolved oxygen, pH and conductivity were measured in each concentration prior to test initiation and at 24-hours. The test was conducted at 25^{0}C in a photoperiod of 16 hours light and 8 hours dark.

TEST DESIGN
Pimephales promelas

The definitive *Pimephales promelas* test was conducted in 300 mL beakers containing 250 mL of test solution. The test was initiated June 28, 2008. Ten *P. promelas* larvae were added to each of two replicate beakers per concentration. Larvae originated from laboratory cultures and were 48-hours old at test initiation. Larvae were fed *Artemia* nauplii prior to test initiation.

A control of two replicate beakers containing ten *P. promelas* larvae each in laboratory water was conducted concurrently with the test. Survival data were statistically analyzed using the Trimmed Spearman-Karber point estimate test to determine the LC50.

RESULTS

Pimephales promelas

The following LC50 value was determined for Oil Spill Eater II (2%):

24-Hour Definitive Test

Conc. (mg/L)	# exposed	# alive	#dead	% survival
Control	20	20	0	100.0
250	20	20	0	100.0
500	20	20	0	100.0
1000	20	20	0	100.0
2000	20	20	0	100.0
4000	20	20	0	100.0
8000	20	1	19	5.0
16000	20	0	20	0.0

Percent Spearman-Karber Trim:	0.00%
Estimated LC50 (mg/L):	**5,856.34**
95% Lower C.L. (mg/L):	5,473.76
95% Upper C.L. (mg/L):	6,265.67

The pH in all solutions was within the organism's tolerance range.

DISCUSSION AND CONCLUSIONS

One LC50 determination was made for Oil Spill Eater II tested at a 2% concentration: 24-hour *Pimephales promelas* LC50: 5,856.34 mg/L. The acute test was conducted from June 28, 2008 to June 29, 2008.

Huther and Associates, Inc.

environmental toxicologists, biologists, consultants

24-HOUR *PIMEPHALES PROMELAS* SURVIVAL

CLIENT: OSE - 28

PROJECT #: 05457

	NUMBER ORGANISMS, 0 HRS		NUMBER ORGANISMS, 24 HRS	
CONC.	A	B	A	B
Con	10	10	10	10
250 mg/L	10	10	10	10
500	10	10	10	10
1000	10	10	10	10
2000	10	10	10	10
4000	10	10	10	10
8000	10	10	9	10
16,000	10	10	0	0
DATE/TIME	mm		mm	
TECHNICIAN	6/28/08 1430		6/29/08 1430	

1156 North Bonnie Brae Denton, Texas 76201 Ph: (940) 387-1026 Fax: (940) 387-1036

OIL SPILL EATER

Test @ 2%

CHEMISTRY MEASUREMENTS

Date	Client	Sample #	ST pH 24hr		ST DO 24hr		Hardness	Alkalinity	Cond. *	~~Ammonia~~	Salinity	Analyst
6/28/05												
			ST	24hr	ST	24hr				pH @ 96hr	D.O @ 96hr	
	LAB CONTROL		7.84	8.60	8.23	7.43			390	7.91	7.92	
	250 mg/L		7.86	8.59	8.22	7.38			389	7.94	8.14	
	500		7.85	8.52	8.20	7.44			387	7.95	8.27	
	1000		7.85	8.48	8.15	7.38			387	7.94	8.26	
	2000		7.86	8.42	8.10	7.53			388	7.93	8.20	
	4000		7.86	8.42	8.15	7.28			390	7.92	8.21	
	8000		7.85	8.31	8.05	7.31			393	7.92	8.05	
	16,000		7.81	8.14	7.87	7.12			399	7.94	8.03	
	LAB WATER CONTROL		7.84				104	64				

TRIMMED SPEARMAN-KARBER METHOD. VERSION 1.5

DATE: JUNE 200 TEST NUMBER: 1 DURATION: 24 H
TOXICANT : OSE II
SPECIES: P. PROMELAS

RAW DATA: Concentration (MG/L)	Number Exposed	Mortalities
.00	20	0
1000.00	20	0
2000.00	20	0
4000.00	20	0
8000.00	20	19
******* 16000.00 BK	20	20

SPEARMAN-KARBER TRIM: .00%

SPEARMAN-KARBER ESTIMATES: LC50: 5856.34
95% LOWER CONFIDENCE: 5473.76
95% UPPER CONFIDENCE: 6265.67

REFERENCE TOXICANTS

TEXAS COMMISSION ON ENVIRONMENTAL QUALITY

NELAP-Recognized Laboratory Accreditation is hereby awarded to

Huther and Associates, Inc.

1156 Bonnie Brae Street
Denton, TX 76201-2421

in accordance with Texas Water Code Chapter 5, Subchapter R, Title 30 Texas Administrative Code Chapter 25, and the National Environmental Laboratory Accreditation Program.

The laboratory's scope of accreditation includes the fields of accreditation that accompany this certificate. Continued accreditation depends upon successful ongoing participation in the program. The Texas Commission on Environmental Quality urges customers to verify the laboratory's current accreditation status for particular methods and analyses.

Certificate Number: T104704233-08-TX
Effective Date: November 9, 2007
Expiration Date: November 30, 2008

Executive Director
Texas Commission on Environmental Quality

Texas Commission on Environmental Quality

NELAP - Recognized Laboratory Fields of Accreditation

Huther and Associates Inc.
1156 Bonnie Brae
Denton, TX 76201

Certificate T104704233-08-TX
Issue Date: 11/9/2007
Expiration Date: 11/30/2008

These fields of accreditation supercede all previous fields. The Texas Commission on Environmental Quality urges customers to verify the laboratory's current accreditation status for particular methods and analyses.

Matrix: Non-Potable Water

Category / Method: EPA 1000

Analytes:	Code	AA	Analytes:	Code	AA
Pimephales promelas	3410	TX			

Category / Method: EPA 1002

Analytes:	Code	AA	Analytes:	Code	AA
Ceriodaphnia dubia	3315	TX			

Category / Method: EPA 1006

Analytes:	Code	AA	Analytes:	Code	AA
Menidia beryllina	3380	TX			

Category / Method: EPA 1007

Analytes:	Code	AA	Analytes:	Code	AA
Mysidopsis bahia	3395	TX			

Category / Method: EPA 2000.0

Analytes:	Code	AA	Analytes:	Code	AA
Aquatic Toxicity, Acute	10341	TX			

Category / Method: EPA 2002.0

Analytes:	Code	AA	Analytes:	Code	AA
Aquatic Toxicity, Acute	10341	TX			

Category / Method: EPA 2006.0

Analytes:	Code	AA	Analytes:	Code	AA
Aquatic Toxicity, Acute	10341	TX			

Category / Method: EPA 2007.0

Analytes:	Code	AA	Analytes:	Code	AA
Aquatic Toxicity, Acute	10341	TX			

Category / Method: EPA 2021.0

Analytes:	Code	AA	Analytes:	Code	AA
Aquatic Toxicity, Acute	10341	TX			

P.O. Box 515429

Dallas, Texas 75251

Ph: (972) 669-3390
Email oseicorp@msn.com

Web www.osei.us

Date June 30, 2008

Toxicity Test Summary for a Ceridaphnia Dubia

Water Flea

The OSEI Corporation performed a toxicity test for a land, water, and airborn based species a Ceriodaphnia Dubia (water flea). The estimated LC 50 for this species even at a higher concentration 20%, than OSE II is applied was 2199.62 which shows that OSE II is also virtually non toxic to bugs as well. The extrapolated value for the LC 50 at OSE II normal application rate of 2% would have been over 4000 mg/l, which shows OSE II is virtually non toxic to water fleas.

Steven Pedigo

Chairman/ CEO OSEI Corporation

OIL SPILL EATER II (2%)
ACUTE PRODUCT TEST

June 2008

24-Hour Acute Toxicity Test Results

Ceriodaphnia dubia

Prepared for:

Oil Spill Eater International, Corporation
13127 Chandler Drive
Dallas, Texas 75243
Tel: 972-669-3390

Prepared by: _____

Bruce Huther
Huther & Associates, Inc.
1156 Bonnie Brae
Denton, Texas 76201
(940) 387-1025 Fax: (940) 387-1036
huther@flash.net

TABLE OF CONTENTS

ACUTE LC50 PRODUCT REPORT

Client	OSEI, Corporation	Project No.	OS457
Sample	2% Oil Spill Eater II	Test Date	June 2008

Results:

24-hr. *C. dubia* LC50:	**> 16,000.00 mg/L**
95% Upper Confidence Limits:	N/A
95% Lower Confidence Limits:	N/A

INTRODUCTION

A product identified as Oil Spill Eater II, Concentrate was delivered to Huther and Associates, Inc. on June 26, 2008. One acute toxicity test was conducted: a static acute 24-hour definitive toxicity test using *Ceriodaphnia dubia* (water flea). Test procedures followed recommended methods contained in "*Methods for Measuring the Acute Toxicity of Effluents and Receiving Waters to Freshwater and Marine Organisms, Fifth Edition*", EPA-821-R-02-012, October 2004.

C. dubia are a freshwater aquatic indicator organism frequently used to evaluate the potential toxicity of a compound or an effluent. The acute toxicity of a compound or effluent is generally measured using a multi-concentration, or definitive test, consisting of a control water and a minimum of five increasing concentrations of product added to control water. The test is designed to provide dose-response information, expressed as the concentration that is lethal to 50% of the test organisms (LC50).

SAMPLE PREPARATION

Oil Spill Eater II was initially prepared for definitive testing by adding the product to distilled, deionized water at a ratio of 50 parts water to 1 part product (2% concentration; stock solution). Seven test concentrations of stock solution were prepared in distilled, deionized water reconstituted to 104 mg/L as $CaCO_3$. The seven concentrations were 250, 500, 1000, 2000, 4000, 8000 and 16,000 mg/L. Dissolved oxygen, pH and conductivity were measured in each concentration prior to test initiation and at 24-hours. The test was conducted at 25°C in a photoperiod of 16 hours light and 8 hours dark.

TEST DESIGN
Ceriodaphnia dubia

The definitive *Ceriodaphnia dubia* test was conducted in 25 mL beakers containing 15 mL of test solution. The test was initiated June 28, 2008. Five C. *dubia* neonates were added to each of four replicate beakers per concentration. Neonates originated from laboratory cultures and were 24-hours old at test initiation. Neonates were fed *Selenastrum capricornutum* prior to test initiation.

A control of four replicate beakers containing five *C. dubia* each in laboratory water was conducted concurrently with the test. Survival data were statistically analyzed using the Trimmed Spearman-Karber point estimate test to determine the LC50.

RESULTS
Ceriodaphnia dubia

The following LC50 value was determined for Oil Spill Eater II (2%):

24-Hour Definitive Test

Conc. (mg/L)	# exposed	# alive	#dead	% survival
Control	20	20	0	100.0
250	20	20	0	100.0
500	20	20	0	100.0
1000	20	20	0	100.0
2000	20	20	0	100.0
4000	20	19	1	95.0
8000	20	20	0	100.0
16000	20	17	3	85.0

Percent Spearman-Karber Trim:	0.00%
Estimated LC50 (mg/L):	**>16,000.00**
95% Lower C.L. (mg/L):	N/A
95% Upper C.L. (mg/L):	N/A

The pH in all solutions was within the organism's tolerance range.

DISCUSSION AND CONCLUSIONS

One LC50 determination was made for Oil Spill Eater II tested at a 2% concentration: 24-hour *Ceriodaphnia dubia* LC50: >16,000.00 mg/L. The acute test was conducted from June 28, 2008 to June 29, 2008.

Huther and Associates, Inc.

environmental toxicologists, biologists, consultants

24-HOUR *CERIODAPHNIA DUBIA* SURVIVAL

CLIENT: OSE 2%

PROJECT #: 05457

	NUMBER ORGANISMS, 0 HRS				NUMBER ORGANISMS, 24 HRS			
CONC.	A	B	C	D	A	B	C	D
CON	5	5	5	5	5	5	5	5
250 mg/L	5	5	5	5	5	5	5	5
500	5	5	5	5	5	5	5	5
1000	5	5	5	5	5	5	5	5
2000	5	5	5	5	5	5	5	5
4000	5	5	5	5	5	5	5	4,
8000	5	5	5	5	5	5	5	5
16,000	5	5	5	5	4,	4,	5	4,
DATE/TIME	6/28/08 1245				6/29/08 1245			
TECHNICIAN	mm				mm			

1445 MacArthur Blvd. Ste. 216 Carrollton, Texas 75007 (972) 242-6844

OIL SPILL EATER

Test @ 28°

CHEMISTRY MEASUREMENTS

Date	Client	Sample #	ST pH 24hr	ST DO 24hr	Hardness	Alkalinity	Cond *	~~Ammonia~~	Salinity	Analyst
6/25/05										
			ST | 24hr	ST | 24hr				pH @ 96hr	D.O @ 96hr	
	LAB CONTROL		7.84 | 8.60	8.23 | 7.43			390	7.91	7.92	
	250 mg/L		7.86 | 8.59	8.22 | 7.38			389	7.94	8.14	
	500		7.85 | 8.52	8.20 | 7.44			387	7.95	8.27	
	1000		7.85 | 8.48	8.15 | 7.38			387	7.94	8.26	
	2000		7.86 | 8.42	8.10 | 7.53			388	7.93	8.20	
	4000		7.86 | 8.42	8.15 | 7.28			390	7.92	8.21	
	8000		7.85 | 8.31	8.05 | 7.31			393	7.92	8.05	
	16,000		7.81 | 8.14	7.87 | 7.12			399	7.94	8.03	
	LAB WATER CONTROL		7.84		104	64				

ACUTE REFERENCE TOXICANT TEST RESULTS

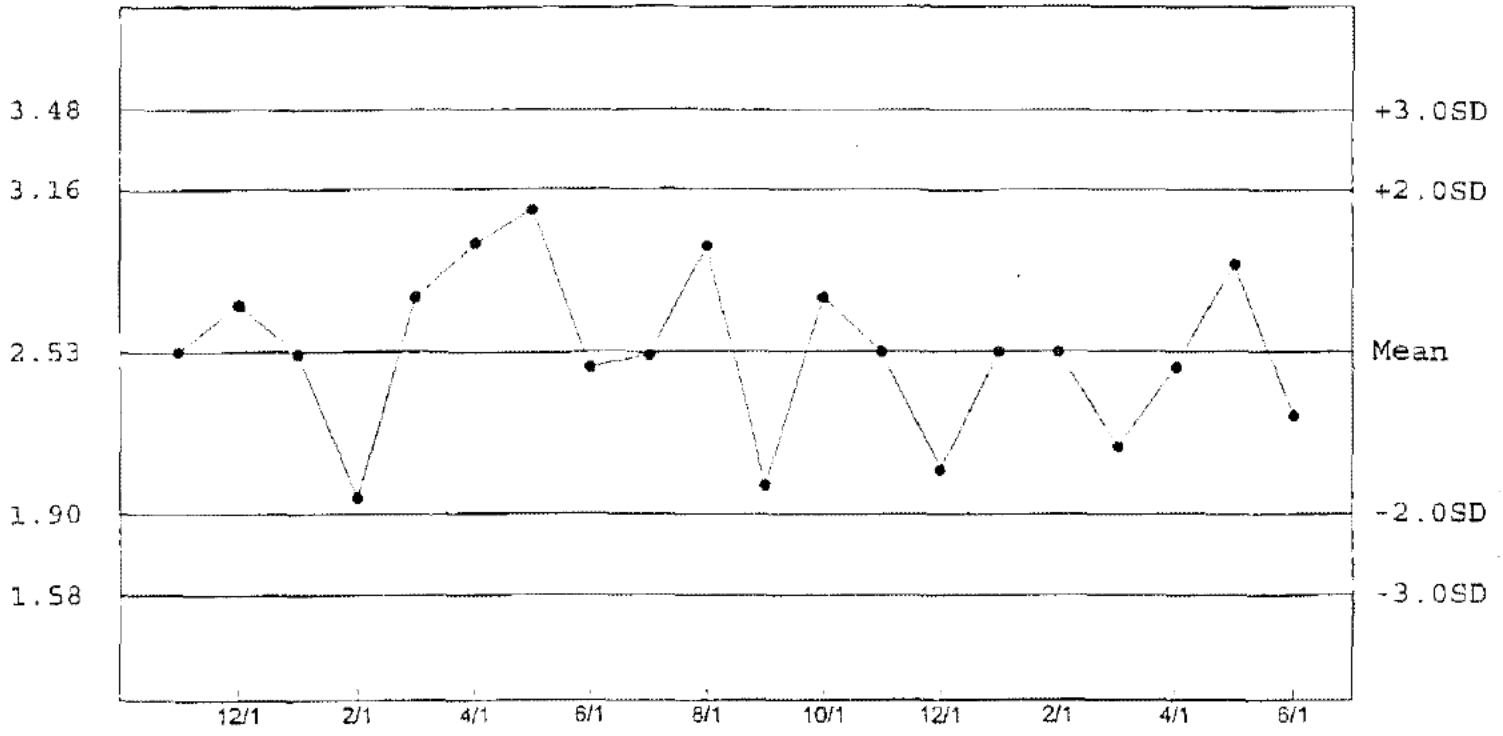
SPECIES:	*Ceriodaphnia dubia*
CHEMICAL:	Sodium Chloride
DURATION:	48-Hours
TEST NUMBER:	6
TEST DATE:	June 2008
STATISTICAL METHOD:	Spearman-Karber

CONCENTRATION (g/L)	NUMBER EXPOSED	NUMBER DEAD
1.0	10	0
1.5	10	0
2.0	10	0
2.5	10	9
3.0	10	10
4.0	10	10

LC50	95% LOWER CONFIDENCE LIMITS	95% UPPER CONFIDENCE LIMITS
2.28 g/L	**2.20 g/L**	**2.37 g/L**

Ref. Toxicant Sodium chloride g/L

Ceriodaphnia dubia LC50



n= 20 Mean= 2.53 SD= 0.32 CV= 12.49% Min= 1.96 Max= 3.08

NELAP CERTIFICATE

TEXAS COMMISSION ON ENVIRONMENTAL QUALITY

NELAP-Recognized Laboratory Accreditation is hereby awarded to

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Executive Director
Texas Commission on Environmental Quality

Texas Commission on Environmental Quality

NELAP - Recognized Laboratory Fields of Accreditation

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Matrix: Non-Potable Water

Category / Method: EPA 1000

Analytes:	Code	AA	Analytes:	Code	AA
Pimephales promelas	3410	TX			

Category / Method: EPA 1002

Analytes:	Code	AA	Analytes:	Code	AA
Ceriodaphnia dubia	3316	TX			

Category / Method: EPA 1006

Analytes:	Code	AA	Analytes:	Code	AA
Menidia beryllina	3380	TX			

Category / Method: EPA 1007

Analytes:	Code	AA	Analytes:	Code	AA
Mysidopsis bahia	3395	TX			

Category / Method: EPA 2000.0

Analytes:	Code	AA	Analytes:	Code	AA
Aquatic Toxicity, Acute	10341	TX			

Category / Method: EPA 2002.0

Analytes:	Code	AA	Analytes:	Code	AA
Aquatic Toxicity, Acute	10341	TX			

Category / Method: EPA 2006.0

Analytes:	Code	AA	Analytes:	Code	AA
Aquatic Toxicity, Acute	10341	TX			

Category / Method: EPA 2007.0

Analytes:	Code	AA	Analytes:	Code	AA
Aquatic Toxicity, Acute	10341	TX			

Category / Method: EPA 2021.0

Analytes:	Code	AA	Analytes:	Code	AA
Aquatic Toxicity, Acute	10341	TX			